Principal Investigator: Cristina De Castro
Grant Title: Chloroviruses encode a novel glycosylation machinery

Abstract

Chloroviruses are large dsDNA viruses that code for up to 400 proteins, with many involved in functions that are not normally found in viruses, as for instance six different putative glycosyltransferases (GTs). The major capsid protein of *Paramecium bursaria* chlorella virus (PBCV-1), the prototype chlorovirus, is extensively N-glycosylated with glycans whose structures do not resemble any structure previously reported in the three domains of life. Disclosure of their biosynthesis is the target of this project.

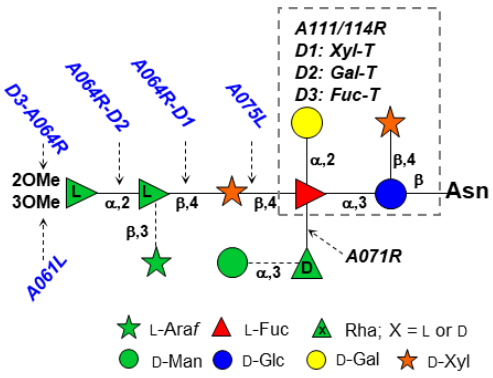
Methods used

All the proteins developed in this study, were expressed in *E. coli* as fusion protein with GSH or His-tag and purified by affinity chromatography. Transferase activity was controlled by using UDP-Glo assay (Promega) or HPLC by using silica C18 columns. All products were characterized with NMR on a Bruker DRX 600 MHz with a z-gradient cryo probe. Bioinformatic analysis was performed with Geneious 11.0.5 software (genetic analysis), while homology searches were run with BLAST.

Results

Experimental work has determined the role of three different proteins and a total of five different activities. The protein A064R consists of three domains, the first domain is a retaining transferase, Mn^{2+} dependent, that attaches β-Rha to any xylose. The second domain is an inverting transferase, cation independent, able to attach a α-Rha to another rhamnose units. Identification of A064R-D2 as a GT has enabled the finding of another transferase in PBCV-1 genome, *a071l*.

The third domain of A064R (A064R-D3), and A061L are the two methyltransferases acting on the terminal rhamnose unit, and A075L is the cation-independent transferase that attaches the distal xylose. Bioinformatic studies have identified in A111/114R three catalytic activities as indicated in the figure. Cloning of the three domains, each separately of together as single protein, has not been successful, so their activity could not be proved. Regarding the way glycans are added to the nascent major capsid protein, our trial to trap the first intermediates with azido-galactose and azido-mannose have not produced results.



PBCV-1 glycan structure with the GTs involved in the glycosidic bond formation. For GTs written in blue the activity has been determined experimentally, while for others, it is only predicted.